ABSTRACT

Diabetes mellitus is one of the major health problems in the world, the incidence and associated mortality are increasing. Inadequate regulation of the blood sugar imposes serious consequences for health. Conventional antidiabetic drugs are effective, however, also with unavoidable side effects. On the other hand, medicinal plants may act as an alternative source of antidiabetic agents. Examples of medicinal plants with antidiabetic potential are described, with focuses on preclinical and clinical studies. A key focus in the research and development of medicinal plants is the identification, isolation, and characterization of bioactive compounds found in plant extracts. Phytochemical studies grounded in ethno pharmacology are regarded as an effective strategy for discovering new chemical compounds with potential as drug candidates. With this approach, the current study focuses on the "Extraction, Isolation & Identification of Antidiabetic Bioactive Compounds from Medicinal Plant in Marathwada Region."

The study will begin with the **selection and authentication of a medicinal plant** based on its traditional use and reported pharmacological potential. The plant material will authenticated by a qualified botanist. The authenticated plant material will then subjected to **extraction using a soxhlet apparatus**, employing a suitable solvent system to obtain a extract. The extract will subject to **phytochemical qualitative & quantitative evaluation.** Subsequently, the extract will evaluated for its **pharmacological activities**, including anti-oxidant and **Antidiabetic effects**, using standard *in vivo* and *in vitro* models. Based on the results, the most potent extract will selected for **fractionation** using chromatographic techniques. The resulting fraction/s will further evaluated for their **Antidiabetic activity** to identify the most active fraction. Finally, the bioactive fraction will subject to advanced analytical techniques, such as LC-MS/GC-MS, to **isolate and identify the phytoconstituent(s)** responsible for the observed **Antidiabetic activity**.

Keywords: Extraction, Fractionation, Isolation, Antidiabetic, Bioactive Compounds, Medicinal Plant, Chromatographic techniques

INTRODUCTION:

Diabetes mellitus According to WHO, the term diabetes mellitus is defined as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunctionand failure of various organs. Diabetes mellitus may have the characteristic symptoms such as thirst, polyuria, blurred vision and loss of weight.[1]

Diabetes is a group of metabolic disease, characterized as increase blood glucose level (BGL), glycosuria, hyperlipidemia, polyuria, polyphagia & polydipsia. It is cause due to reduction in the level of insulin in blood or pancreas does not produce sufficient insulin. High BGL damages the neurons and blood vessels, this lead to micro-vascular complications such as nephropathy, retinopathy & neuropathy, while macro vascular complications result into stroke & heart failure. In other words, a condition associated due to deficiency of the insulin.[1,2]

The cause of diabetes is may be due to one of this mechanism:[3]

1. Insufficient production of insulin by the pancreas (may be due to abnormality in gluco-receptor of β-cells or relatively due to β-cells deficiency.
2. Non sensitivity of peripheral tissues to the insulin.
3. High level of hyperglycemic chemical mediator likes glucagon and so on. Obesity also causes insulin deficiency.
4. Formation of highly reactive oxygen species due to hyperglycemia. This will lead to many degenerative disorders including diabetes mellitus.

The disease spread over the world and moving all parts of various communities. The most common form of disease was found as Type-II diabetes mellitus. The symptoms which is associated with the diabetes mellitus includes frequent urination, shivering or deadness in the hands or feet, exorbitant thirst, feeling drowsy, loss body weight, extraordinary appetite, abrupt vision changes might be obscured vision, dehydrated skin, injuries that are delayed to recuperate and more diseases than expected and other more symptoms.

Regulation of insulin: Insulin has 51 amino acids within two chains of polypeptide. Normally 1U insulin is discharged every hour from human pancreas. Its metabolic role is to transfer glucose molecule into the cell of body through trans membranous transport mechanism. Glycogen synthesis and skeletal muscle also required insulin. Conversion of glucose to triglycerides and for synthesis of nucleic acid and proteins. It acts by binding to

the insulin receptors cells. The synthesis and release of insulin is regulated by chemical, hormonal and neural mechanism. A defect in the biosynthesis or release may lead to deficiency or insufficient release to meet the demand of body. The steps involved in biosynthesis process as follows: I. Insulin is synthesized from pre-proinsulin (110 amino acid) as single peptide chain in pancreatic islet of β-cell, out of which 24 amino acids are evacuated to deliver.[4]

Proinsulin

Proinsulin (86 amino acid) is shifted to the golgi apparatus where C-peptide (35 amino acids) is split off by a proteolytic enzyme. C-peptide and insulin are put in storage inside the cell where they are secreted in membranous sacs acquired from the golgi membranes into the cytoplasm where insulin is transformed into zinc-insulin crystals.[4]

Fate of insulin [3,4]

Insulin secretion regulated by chemical mediators, hormones and neurons:

***Chemical mediators*:** The raise in intracellular Ca2+ causes exocytotic release of Insulin from storage granules of β-cells of pancreas which have a glucose sensing detector system which permits entry of glucose into beta cells.

***Hormones*:** For release of insulin from granules various hormones were responsible such as growth hormone, corticosteroids, thyroxin adjusts insulin discharge in response to glucose. It has been appeared that insulin release obstructed with prostaglandin (PGE). Somatostatin hinders arrival of both insulin and glucagon. Glucagons bring out arrival of insulin just as somatostatin. Insulin restrains glucagon release.

***Neuronal*:** The β-cell of islets is highly provided by sympathetic and vagal nerves. Adrenergic α2 receptor initiation declines insulin secretion by inhibition of β-cell adenylyl cyclase. Insulin secretion increase by stimulation of β-cell adenylyl cyclase, due to activation of adrenergic β2 receptors. Acetylcholine stimulates cholinergic muscarinic receptor which causes increase in conc. of Ca2+ in the β-cell lead to exocytosis release of insulin from β-cell. Extracellularly insulin is distributed throughout the whole body. Insulin is destroyed in the g.i.t. (gastrointestinal tract) due to their peptide nature whenever given orally. Insulin is metabolised in liver and in lesser extent in muscles & kidney. The 50 % of insulin in blood or body from pancreas is inactivated through liver by first passage mechanism. In this manner, the disulphide bond is reduced to separate A and B chains of amino acids, during the biotransformation. The separated chains are additionally separated into the various constituents of amino acids.

Types of diabetes mellitus [2,3,]

Type-I diabetes mellitus

A complete deficiency of insulin hormone or very lesser amount of insulin to regulate blood sugar level correctly is the main cause. The deficiency of insulin may be due to one of reasons that destruction of β-cell in pancreatic islets cell due to autoimmune disorder. The metabolism of carbohydrate, proteins and fats is altered an estimated 5% to 10% of those with type-1 diabetes. In spite of the fact the condition can show up any age. Beginning of type-1 diabetes is the most widely recognized in kids and young grown-ups this is the reason it was earlier called juvenile diabetes mellitus. The deficiency of insulin leads to various complications such glucose was not used as source of energy by body, though there was increased level of glucose in blood and lead increased hunger. High level of glucose also causes frequent urination than usual, it causes excessive thirst. The insulin producing beta cells were destroyed after diagnosis of condition with in 5 to 10 years. This condition was occurred at any stage of age however it, for the most part, begins in individuals younger than 30 years.

Type-II diabetes mellitus

In this condition, body does not produce required quantity of insulin for proper metabolism of glucose. Insulin does not work effectively may be due to does not meet the required amount of demand for metabolism of glucose as problem in their synthesis. Insulin is vital requirement of glucose for transmembrane passage to inside the most the cells of body. It was normally found in grown-ups beyond 40 years of age, may be due to increased body weight i.e. obesity but it is actually being diagnosed at early ages also and is presently much of the time found in kids too. Originally in this disease, insulin is secreted but very less quantity which cannot work properly. This will lead to condition called insulin resistance. Insulin is required by glucose to get inside the cell, unless the body cannot transform glucose into energy. If excess amount of glucose in the blood circulation it may be secreted in the urine by kidney. This high amount of glucose in blood called hyperglycaemia. Type-II diabetes generally happens gradually or steadily. During diagnosis, majority of the individual with type-2 diabetes was found that they have high weight than usual.

Gestational diabetes mellitus

It is developed during pregnancy. It occurs more often in person that has previous family background of diabetes. Mostly, it vanishes after delivery. In this condition pregnant women have high amount of insulin, but the action is blocked due to certain hormones of placenta, this condition leads to insulin resistance. Placenta has major role for supply of food, water

and other nutrients to the growing foetus and furthermore produces hormones to maintain pregnancy. Hormones of placenta like cortisol, estrogens and Human Placental Lactogen (HPL) have inhibitory action on the release of insulin hormones or blocking action on insulin. As the placenta grows there is more hormones produce and begins to develop insulin resistance. The production of insulin from βcells of pancreas is reached to their maximum level but still not enough to overcome the action of placental hormones, this will lead to gestational diabetes.

Diabetes insipidus

It is the conditions were the kidney is incapable to retain water, that was responsible for repeated urination and develop excessive thirst. It is exceptional condition occurs when kidney does not reabsorb water molecule. The antidiuretic hormones (ADH) directed to save water. At the point when the kidney doesn't react to ADH in diabetes insipidus, the situation is called nephrogenic diabetes insipidus. The fundamental indications are unnecessary urination and extraordinary thirst. Thirst encourages the patients to drink more water to make up for water lost in the urine. The hypothalamus or pituitary organ when harm due to medical procedure, infectious disease or head injury prompts to Central diabetes insipidus.

Treatment of diabetes mellitus[5,6]

The present treatment of DM is focused on controlling and lowering blood glucose levels in the vessel to a normal level. The mechanisms of both modern medicines and traditional medicines to lower blood glucose concentration are:

(1) Stimulate beta-cell of the pancreatic islet to release more insulin;

(2) Inhibit the action of hormones which increases blood glucose concentration;

(3) Increase the sensitivity of insulin receptor site;

(4) Inhibit hydrolysis of glycogen in liver;

(5) Enhance the use of glucose in tissues and organ.

Currently, there are six main classes of modern medicines used all over the world for controlling blood glucose levels and two classes of injections. The tablets are known as biguanides (metformin), sulfonylureas, thiazolidinediones (glitazones), meglitinides (glinides), alpha-glucosidase inhibitors and DPP-4 inhibitors. The classes of medications given by injection are incretin mimetics and insulin. Mechanisms of these medications have been reported. However, most modern drugs have many side effects and adverse effects, causing some serious medical problems during medication processing. Metformin is one medicine of biguanides which can inhibit the production of glucose molecules in the liver of human and increase insulin sensitivity. However, metformin also causes some main side

effects such as gastrointestinal on the initial state, including dyspepsia, nausea and diarrhea. Metformin should be avoided in those with severely compromised renal function, de-compensated heart failure, severe liver disease other serious medical problems. The beneficial effects of thiazolidinediones in diabetes treatment are to improve the sensitivity of insulin, decrease insulin resistance and also decrease cardiovascular risks. However, the most common adverse effects of thiazolidinediones are weight gain and fluid retention, leading to peripheral edema and heart failure. The drugs were avoided to use for patients in similar situations, including heart failure and severe liver problems. Rosiglitazone may have cardiovascular risks, and increase the risk of a heart attack. Pioglitazone has not been available in some countries due to concerns about an increased risk for bladder cancer.

Besides modern medication, traditional medicines have been used for a long time and play an important role as alternative medicines. According to WHO, a plant-based traditional system of medicine is still the chief support of about 75–80% of the world population mainly in developing countries having a diversity of plants. Traditional medicines are usually the first choice for primary healthcare of patients in developing countries because of better cultural acceptability, better compatibility with the human body and lesser side effects than modern therapies. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies. More than 400 plant species having hypoglycemic activity have been available in the literature, however, investigating new antidiabetic drugs from natural plants has still been attractive because they contain phytoconstituents that demonstrate alternative and safe effects on the treatment of diabetes mellitus. Most plants contain bioactive components, such as phenolics, glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that have been improved as having antidiabetic activities. [6,7]

Sovarious medicinalplantsareavailable intraditionalsystemfor itseffectiveness inDiabetic but systematic studies ofmanyplants yet to be explored. Manyplants just evaluated fortheir preliminary activity but not for their extensive screening including that for bioactive guided isolation and characterization. Accordingly, the present study is aimed for,

“Extraction, Isolation & Identification of Bioactive Compound from Medicinal Plants for its Antidiabetic activity”

AIM & OBJECTIVES OF RESEARCH

Present study is aimed for **"Extraction, Isolation & Identification of Bioactive Compound from Medicinal Plants for its Antidiabetic activity"**

With objectives-

1. Phytochemical investigation & standardization of selected Medicinal Plant & its extracts.
2. Pharmacological evaluation of plant extracts and fractions for its anti-diabetic activity
3. Isolation & identification of bioactive phytoconstituent/s from the plant extracts & fractions

4.Toxicity study of extract/s to obtain safety data

PLAN OF WORK

1. **Literature review**
2. **Collection, identification and authentication of selected plant material**
3. **Processing of plant material**
4. **Pharmacognostic evaluation of plant material**

   Morphological evaluation, microscopic evaluation and powder characteristics
5. **Standardization of plant material**
   - Extractive Value & Ash content
   - Acid &Water-soluble ash
   - Foreign matter
   - LOD
6. **Extraction of selected plant material**
7. **Phytochemical screening of selected plant extract/s for**

   a) Phytochemical Qualitative analysis
   - Chemical tests of Phytoconstituents
   - TLC study

   b) Phytochemical Quantitative analysis
   - Total Phenolic Content
   - TotalFlavonoid Content
8. **Pharmacological screening of plant extract/s for-**

   a) *In-vitro*Antioxidant study

   b) Safe dose calculation by acute oral toxicity studies and repeated dose toxicity study

   c) *In-vitro* Antidiabetic activity

   d) *In-vivo*Antidiabetic activity
9. **Isolation & identification of Antidiabetic bioactive constituent**

   i. Selection of extract

   ii. HRLC-MS Study of selected extract

   iii. Fractionation

   iv. Phytochemical evaluation of fraction/s

   v. Pharmacological screening of fraction/s

   vi. Identification of phytoconstituents
10. **Interpretation of data and its presentation**

RATIONALE OF STUDY:

According to the International Diabetes Federation (IDF) report, there are 451 million (age 18–99 years) people with diabetes worldwide. These figures were expected to increase to 693 million) by 2045. It was estimated that almost half of all people (49.7%) living with diabetes are undiagnosed. Moreover, there were an estimated 374 million people with impaired glucose tolerance (IGT) and it was projected that almost21.3 million live births to women were affected by some form of hyperglycemia in pregnancy. In 2017, approximately 5 million deaths worldwide were attributable to diabetes in the 20–99 years age range. Several studies reported the prevalence of diabetes mellitus complications varying from 20 to 90.5%. Previous studies in Ethiopia revealed that hypertension, visual disturbance, nephropathy, and neuropathy were the highest four chronic complications diagnosed in diabetic patients.

Generally, there are four classifications of diabetes mellitus such as Type I DM, Type II DM, gestational GDM, and specific types of DM (drug- induced diabetes, latent autoimmune diabetes in adults, cystic fibrosis diabetes, monogenic diabetes. Oxidative stress is believed to be the basic cause of tissue damage, organ dysfunctions, and cellular injury

Herbal medicine, as a major part of traditional medicine, has been used in medical practice since antiquity and is a common element of ayurvedic, homeopathic and naturopathic medicine. World Health Organization (WHO) notes that 74% of the plant derived medicines are used in modern medicine, in a way that their traditional modern application directly correlates with their traditional use as herbal medicines by native cultures. WHO estimates that 65% of the world's population uses traditional medicines as their primary form of health care and about 85% of traditional medicines involve the use of herbal preparations. The global market of herbal drugs at present is approximately $ 600 billion.

According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3%. Despite the availability of a wide range of drugs, the relief offered by them is mainly symptomatic and short lived. Moreover, these drugs produce side effects. Management of diabetes without side effects is still challenge to the medical community. There comes the importance of Natural remedies. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary

health care. Assessing the current status of health care system in adequacies of synthetic drugs is likely to be more glaring in the coming years. It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine

Isolating and identifying the bioactive compounds from plants helps the discovery of novel drugs that could serve as the foundation for safer, more effective antiasthmatic treatments. Modern pharmacological and toxicological profiling of these constituents ensures their efficacy and safety. The increasing awareness of the side effects and limitations of synthetic drugs has led to a growing demand for natural and herbal treatments. Researching traditional remedies validates their use and integrates them into mainstream healthcare.

Materials and Methods

- **Plant Material:**

  The selected plant part/s will be collected from known sources and authenticated by an expert botanist.

- **Processing of Plant Material:**

  The collected crude drugs will be processed according to official standards, including steps such as drying, grinding, and sieving.

- **Standardization of Plant Material:**

  Phytochemical examinations will be conducted using standard methods, including the estimation of extractive value, acid and water-soluble ash, foreign matter, and loss on drying (LOD) etc.

- **Pharmacognostic Evaluation of plant material:**

  The parts of the selected plant/s will be subjected to Pharmacognostic evaluation, including morphological evaluation, microscopical evaluation, and powder characteristics etc.

- **Extraction of Plant material:**

  The powdered form of the selected plant material will undergo extraction, with the choice of solvent and extraction method determined based on the chemical constituents of the selected plant parts and the nature of those constituents.

- **Phytochemical Screening of selected plant extract/s for**

  Qualitative analysis will be conducted to detect phytoconstituents, which will then be confirmed using suitable chromatographic techniques.

  Quantitative analysis will be performed to determine the total polyphenols, total flavonoids etc.

- **Pharmacological screening of plant extract/s**

  The extract/s of the selected plant parts, either individually or in combination, will be evaluated for

  I.*In-Vitro*Antioxidant study.

  In vitro antioxidant activities for the extract/s will be carried out using appropriate *in-vitro* antioxidant model like; DPPH assay

II. Safe dose calculation:

The safe dose calculation of plant extract/s for efficacy evaluation will be conducted in accordance with OECD Guidelines for Acute Toxicity Study: Class Method(423).

Repeated dose oral toxicity study

Repeated dose oral toxicity study of plant extract/s will be carried out as per OECD Guidelines for 'Repeated dose oral toxicity study: Class Method (407)

III. Antidiabetic activity:

A. *In-vitro* antidiabetic activity

i. α-amylase Inhibition Activity

ii. α-glucosidase Inhibition Activity

B. ***In-vivo* antidiabetic activity**

i. Alloxan-induced diabetic model

ii. Streptozocin-induced diabetic model

Any one models from the above list will be used to study the anti-diabetic activity of various plant extract/s.

- **Isolation & identification of antidiabetic bioactive constituent**

i. Selection of extract/s

After preliminary phytochemical and pharmacological evaluations for antioxidant, anti-inflammatory, and antidiabetic activities, the most potent extract/s will be selected for further studies.

ii. HR-LCMS Study of selected extract:

The selected extract/s will be subjected to HR-LCMS studies to confirm the presence of various phytoconstituents in extract/s.

The abundant bioactive phytoconstituents will be subjected for further study**.**

iii. Fractionation:

Further as per the results of HR-LCMS , the extract/s will be subjected to fractionation for purification & isolation of bioactive phytoconstituentsS & the fractionation will be done by using solvent fractionation(A technique used to separate components of a mixture by exploiting differences in their polarity)

iv. Phytochemical evaluation of fraction/s:

The resulting fraction/s will undergo qualitative analysis, including identification tests, and HPTLC studies.

v. Identification of phytoconstituents

The phytoconstituent/s will be identified by using LC-MS / GC-MS spectroscopic techniques to confirm presence of bioactive constituent in fraction/s.

vi. Pharmacological screening of fraction/s:

Finally, the isolated phytoconstituent/s or fraction/s will be subjected to pharmacological confirmation of their antidiabetic activity by *in-vitro*&*in-vivo* antidiabetic model.

YEAR WISE PLAN OF WORK:

Sr. No.	Work	Period in months
1.	Literature survey	06
2	Plant collection and preliminary screening	03
3	Extraction of plant material	05
4	Phytochemical evaluation	04
5	Pharmacological screening of extracts	08
6	Isolation and identification of Phytoconstituents/s	04
7	Pharmacological screening of fraction	02
8	Compilation of data & Thesis writing	04
	Total duration of project	**36 months**

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Date:

Place: Nanded

Name & Signature of Research Scholar **Name & Signature of Supervisor**

Mr. Prashant V. Sawale **Dr.Ashish B Roge**

LITERATURE SURVEY

Extensive literature survey reveals numbers of medicinal plants are claimed in traditional system for its effectiveness in diabetic so many plants just evaluated for its activity.

Literature Review on Antidiabetic activity

Scientific name	Family	Local name	*In vivo* Test	*In vitro* Test	Plant part	Effect	Reference
Calpunia aure	Fabaceae	Ligita	Normoglycemic, OGTT and STZ induced DM	–	Leaf	↓BGLatalldosesofthe extract($P<0.05$)at days 7,14,and21.Antidiabeticactivity($P<0.05$)exhibitedby400mg/kgcomparedto100mg/kg.	14
Justicia schimperiana	Acanthaceae	Smiza	Normoglycemic, OGTT and STZ induced DM	–	Leaf	Showed significant tolerance ($P<0.05$) at 1 and 2 h ↓BGL ($P<0.05$) at 4 h in normoglycemicmice.↓BGL($P<0.05$)at400 mg/kg extract at 2, 3, and 4 h of treatment indiabeticmice.	15
Terminalia brownie	Combrefaceae	Abalo	Normoglycemic, OGTT and STZ induced DM		Stem bark	↓HyperglycemiawithOGTTbythecrude extract at a dose of 500 mg/kg($P<0.01$), 750 ($P<0.05$) after 60 min, and 750mg/kg($P<0.01$)after120min↓BGL($P<0.01$)withethylacetateandaqueousfractionsat500 mg/kgindiabeticmodel.	16
Croton macrostachys	Euphorbiaceae	Bisana	Normoglycemic, OGTT and STZ induced DM	–	Root	↓Hyperglycemia by 300 mg/kg compared to 100 ($P<0.001$) and 200 mg/kg ($P<0.01$) in diabeticmice. ↓BGLinOGTTatdoses of100 ($P<0.01$),200($P<0.001$), and300mg/kg($P<0.001$)after60,90,and120minofglucose loading.	17
Indigofera spicata	Fabaceae	Notstated	Normoglycemic, OGTT and Alloxan-induced DM	–	Leaf	↓BGLat200and400mg/kginnormoglycemic mice ($P<0.05$). ↓BGL ($P<0.05$) in only400mg/kgexposed groupsat the120minofpostexposureinOGTTmodel. ↓BGL($P<0.05$)atalldoses oftheextractat4, 6, and 10 h on diabetic mice.	18
Otostegia	Lamiaceae	Tinjut	Normoglycemic,	–	Leaf	↓BGLs at 200 mg/kg extract in the hypoglycemic and OGTT models.	19

integrifolia			OGTT and STZ induced DM			↓FastingBGL(P<0.001)at100and200mg/kgdoses at 4 h in diabetic mice.	
Caylusea abyssinica	Resedaceae	Akorarach	Normoglycemic, OGTT and STZ induced DM	–	Leaf	↓BGLby100(P<0.05)and300 mg/kgextract (P <0.01) starting from the 3rd h, and by 200 mg/kg (P <0.001) as early as the 2nd h inDiabeticmice. ↓BGLby100 mg/kgextract(P<0.01)at120minand200mg/kg(P<0.001) at 60 min in OGTT.	20
Rubus Erlangeri	Rosaceae	Notstated	Normoglycemic, OGTT and STZ inducedDM	α-amylase	Leaf	↓BGL(P<0.05)attheextract(100,200,and 400 mg/kg) 14 days.	21
Lens culinaris	Leguminosae	Notstated	Normoglycemic, OGTT and STZ induced DM	–	Seed	↓BGLatalldosesofthe extract(P<0.05)at days 7,14,and21.Antidiabeticactivity(P <0.05)exhibitedby400mg/kgcomparedto100 mg/kg.	22
Aloe camperi	Asphadelaceae	Ere	OGTTand Alloxan-induced DM	–	Leaf	Showed a significant (P<0.001) reduction of BGLascomparedtothediabeticcontrolgroup.	23
Capparis tomentosa	Capparaceae	Gumero	Alloxan-induced DM	–	Root	↓BGLatalldosesofthe extract(P<0.05)at days 7,14,and21.Antidiabeticactivity(P <0.05)exhibitedby400mg/kgcomparedto100 mg/kg.	24
Catha Edulis	Celastracea	Khat	Normoglycemic, OGTT and STZ	–	Leaf	↓FastingBGLfrom223.7± 27.6to106±18.2 mg/dl, at the end of study (P <0.05).	25

Literature Review on analytical technique for Isolation & Identification

Sr no.	Plant name	Analytical technique	Conclusion	Reference
1.	*Hibiscus asper*	GC-MS	The GC-MS analysis revealed the presence of twenty-three bioactive compounds which include 9,12,15-octadecatrien-1-ol, n-Hexadecanoic acid, octadecatrienol acid, methyl palmitate, and phytol.	26
2.	*Vaccinium bracteatum*	HPLC	The analytical technique isolate the vaccinoside, which had been the only compound isolated extract, along with vanillic acid and protocatechuic acid .	27
3.	*Albizia coriaria*	FTIR, TLC, GC-MS	The analytical studies found the presence of triterpenoid Lupeol, lupenone, betulinic acid, botulin etc	28
4.	*Teucrium polium*	HR-LCMS	The analytical studies identified the 13R-hydroxy-9E,11Z octadecadienoic acid, dihydrosamidin, valtratum, and cepharantine	29
5.	*Myristica dactyloides*	HR-LC-MS	results prove the presence of Myoinositol, Malabaricone B, Malabaricone C, Malabaricone D, and 1-(2,6-dihydroxyphenyl)-9-(4-hydroxy-3-methoxyphenyl) nonan-1-one previously reported from *M. dactyloides*, along with promising chemical signatures like Monotropein, Austrobailignan,Fragransol B, Guaiacin, Myricanone, Nectandrin A etc	30
6.	*Saracaasoca*	LC-MS	Showed the presence of Flavonoids such as quercetin, naringenin, epiafzelechin are found to be higher than all other compounds responsible for elevated response to various infections and damage	31